[SEQ CHAPTER \h \r 1]**Primary Reviewer:**	Cassandra Kirk, Ph.D., Biologist, Emerging Technologies Branch	**Date:** 11/25/2019
	[SEQ CHAPTER \h \r 1]	
[SEQ CHAPTER \h \r 1]Secondary Reviewer:	Chris Wozniak, Ph.D., Biotechnology Special Assistant, OPP/BPPD	**Date:**

DATA EVALUATION RECORD

[SEQ CHAPTER \h \r 1]**REQUIREMENT:** EPA OCSPP 850.2100

TEST MATERIAL (PURITY): *Aedes aegypti* OX5034

SYNONYMS: OX5034

CITATION: Tetracycline-Repressible Transactivator Protein Variant (tTAV - OX5034 OX5034) and Related Genetic Material from OX5034 *Aedes aegypti*: Request for Waiver from the Avian Acute Oral Toxicity Test. Volume 11, EUP Submission; MRID 50889410, July 16, 2019.

SPONSOR: Oxitec Ltd, 71, Milton Park, Abingdon, Oxfordshire, OX14 4RX United Kingdom

AUTHOR: Oxitec Ltd.

TEST SITE: Not applicable

COMPLIANCE: Good Laboratory Practice Standards, 40 CFR Part 160, are not applicable to this document. This study is a request to waive the data generation requirements for an Avian Dietary Toxicity study.
This DER does not contain FIFRA CBI.

EXECUTIVE SUMMARY:

The developer of the male-sterile *Aedes aegypti* OX5034 mosquito, Oxitec Ltd., requests a waiver for performing an avian dietary toxicity study through application of rationale outlining the reasons that such a test is unnecessary. Oxitec has indicated that the Yellow Fever mosquito, *Aedes aegypti*, 1) are primarily found in urban areas and have little or no interaction with avian species in natural ecosystems; 2) *Aedes aegypti* are a negligible component of the diet of insectivorous birds; 3) threatened and endangered avian species typically do not inhabit urban areas and will not be exposed to OX5034 mosquitoes; 4) OX5034 mosquitoes will not feed on birds; and 5) OX5034 mosquitoes have not caused adverse effects when consumed by non-avian species. The reviewer agrees with the above statements 2) through 5) but disagrees with

statement 1) because urban areas are a part of a larger ecosystem and there are non-target species, including avian species, present.

Although OX5034 has not been tested on avian species, feeding studies on fish and invertebrates have shown no evidence of any adverse impacts. Studies demonstrate the tTAV-OX5034 and DsRed2-OX5034 proteins do not share homology with known toxins or allergens based on bioinformatics analysis and therefore are considered to be non-toxic and non-allergenic (MRID 50889420). In addition, OX5034 *Aedes aegypti* have not caused adverse effects when consumed by non-avian species. While tTAV-OX5034 can exhibit toxic effects within cells, specifically in the nucleus where it is expressed, this is not observed following oral consumption of this protein as the digestive process degrades the integrity of the protein and uptake of this intact protein into cells and nuclei is highly unlikely.

PURPOSE OF THE STUDY:

The OCSPP guideline 850.2200 is intended for use in developing data, specifically both a median lethal concentration (LC50) and slope of the concentration-response, on the dietary toxicity to young northern bobwhite (*Colinus virginianus*) and mallard (*Anas platyrhynchos*) of chemical substances and mixtures ("test chemicals" or "test substances") subject to environmental effects test regulations. While the study is specifically designed to allow calculation of the LC50, the study can be used to obtain information regarding sublethal effects which are used in Agency evaluations. This guideline prescribes specific guidance for the testing of northern bobwhite and mallard, which are the Agency's preferred test species. The Agency will use these data to assess acute hazard to birds. The use of a test based primarily on lethality is justified because it presents or insures a consistent, unbiased endpoint for assessment purposes and has unambiguous ecological relevance to adverse effects. This submission seeks to provide suitable rationale to alleviate the need for conductance of this study through data generation.

CLASSIFICATION: ACCEPTABLE

I. MATERIALS AND METHODS:

A. GUIDELINE FOLLOWED: OCSPP Guideline 850.2200

Deviations from guideline: Waiver request.

B. MATERIALS:

1. Test Material: *Ae. aegypti* OX513A-tTAV / DsRed2-OX5034

Control Substance: N/A

2. **Test Organism:**

Species (common and scientific names): N/A

Age at study initiation: N/A

Number of test individuals /Sex: N/A

Strain/Source: OX5034 was developed via standard micro-injection methods (Morris, 1997; Jasinskiene et al., 1998),by injecting a combination of pOX5034 plasmid DNA (containing the tTAV-OX5034 and DsRed2-OX5034 genetic material) and *piggyBac* mRNA as the source of transposase, into *Aedes aegypti* mosquito eggs of an arbovirus free Latin American wild-type strain (originating from Chiapas, Mexico, and held in Oxitec labs since 2006). The transposase mRNA provides a source of *piggyBac* transposase, to allow the rDNA construct to be integrated into the germline of *Aedes aegypti*. The non-autonomous transposon has no endogenous source of transposase in mosquitoes and has had no further translocation. The resulting OX513A line has been maintained in a continuously cycling insect colony for the equivalent of over 27 generation equivalents. Sterile males, homozygous for the two transgenes, are to be released for population suppression; a very low number of homozygous (tTAV-OX5034/ DsRed2-OX5034). When male OX5034 *Aedes aegypti* homozygous for the conditional female-specific self-limiting gene (carrying two copies of the gene) are released into the environment and mate with wild *Aedes aegypti* females, their offspring inherit a single copy (so are hemizygous) of the self-limiting gene. The self-limiting gene kills only female offspring (carrying one copy of the self-limiting gene), which die at early larval stages of development, while hemizygous males will survive to pass the OX5034 genes on further. Hence the OX5034 mosquito can be considered to be a species-specific female larvicide for *Aedes aegypti*.

BPPD Comments:
Aedes aegypti is known to frequent households and associated habitat in close proximity to buildings inhabited by humans. This is in part a consequence of their preference for human blood as a source of nutrients to supply developing eggs (Scott et al., 2000; Harrington et al., 2001; Ponlawat and Harrington, 2005) and utilization of containers for oviposition and larval development. *Ae. aegypti* do, however, utilize other mammalian and avian sources for bloodmeals, including dogs, cats, cattle, horses, swine and chickens (Jansen et al., 2009; Barrera et al., 2012). Local climatic conditions and geography (e.g., degree of urbanization) may affect the distribution of *Ae. aegypti* in a particular season (Scott et al., 2000). There are domestic, sylvan and peridomestic forms of *Ae. aegypti* which predominately reside in urban / household, forested, or environmentally modified areas (e.g., coconut groves, farms), respectively; however, not all of these forms may exist in any one area or country (Tabachnik et al., 1978).

Given the potential for some limited feeding on avian species by a small number of inadvertently released female OX5034 mosquitoes, it is important to examine the possible impacts of these species serving as a source of a bloodmeal. The production process for the OX5034 mosquitoes

includes a screening for arboviruses known to be vectored by *Ae. aegypti*. Detection of any of these arboviruses in the breeding stock or production population would require destruction of all of the mosquitoes involved and an evaluation of possible sources for such a viral contaminant. In a worst-case scenario, these OX5034 females would function similarly to those present in the wild population, albeit without the potential to vector a pathogenic virus. The presence of the tTAV and DsRed2 proteins, if present in saliva, would not contribute a risk to the potentially affected avian species as neither are known to be toxic or share homology with known toxins.

Concerns regarding oral consumption of OX5034 mosquitoes by avian species similarly would not be considered as a significant risk due to a lack of plausible toxicity to these species via uptake during normal digestive processes. Mosquitoes tend to make up a very small portion of the avian diet in insectivorous birds as well, reducing the net exposure to OX5034. Digestive processes would be expected to degrade proteins, including tTAV-OX5034 and DsRed2-OX5034, as part of the typical acid and enzymic hydrolysis of proteins.

The waiver request is appropriate considering the lack of toxicity and minimal exposure of avian species to released OX5034 mosquitoes.

CONCLUSION: ACCEPTABLE

REFERENCES:

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